Investigation of the Toxic and Teratogenic Effects of GRAS Substances
to the Developing Chicken Embryo
MANNITOL

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Protocol:

Mannitol was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by the two routes at two stages of embryonic development: via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (1, 2).

Groups of 10 or more eggs were treated under these four conditions at several dose levels until a total of ninety to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. All hatched chicks and non-viable embryos were examined carefully for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively (the milligrams per kilogram figure is based on an average egg weight of fifty grams). Column 3 is the total

number of eggs treated. Column 4 is the percent mortality i.e. total non-viable divided by total treated eggs. Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, ataxia or other nerve disorders. Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent treated eggs and the untreated controls.

The mortality data in Column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (3). The results obtained are indicated at the bottom of each table.

The data of Columns 4, 5, and 6 have been analyzed using the Chi Square Test for significant differences from the control background. Each dose level is compared to the control value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

At hatchings, 3 chicks were removed at random from each level including control for skeletal clearing, weighing and fixing of bursa, spleen, liver and kidney. Tissues were processed, blocked in paraffin, sectioned, affixed to slides, and stained. Later these sections were examined for internal damage to the tissues.

Discussion:

Mannitol was tested at dose levels between 3.75 and 200 mg/kg for all four conditions of the test. For air cell treatment at 00 hours, the estimated LD-50 is 720.465 mg/kg (36.02 mg/egg). For 96 hours air cell treatment, the slope of the line was not significantly different ($p=0.05$) from zero and an LD-50 estimate could not be made.

For yolk at 00 hours and 96 hours treatment, the estimated LD-50 is 140.17 mg/kg (7.01 mg/egg) and 222.21 mg/kg (11.11 mg/egg), respectively.

Significantly higher mortality rate was observed in all the four treatments when the test dose was 200 mg/kg. Air cell treatment at 0 hours and yolk treatment at 96 hours were more susceptible to Mannitol than the other two treatments. In the above two treatments, 100 mg/kg dose level produced significantly higher mortality rates when compared to solvent.

There were no terategenic effects observed in all the four test conditions employed.

References:

1. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5., 760-770.

2. Verrett, M. J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1003-1006.

3. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendic I.

MANNITOL
AIR CELL 0 HOURS

DOSE mg/egg	DOSE mg/kg	Number of Eggs	Percent* Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	100	40.00*	0.0	0.0
5.00	100.00	100	26.00*	0.0	0.0
0.75	15.00	100	19.00	0.0	0.0
0.375	7.50	100	12.00	0.0	0.0
0.1875	3.75	100	6.00	0.0	0.0
Water		100	9.00	0.0	0.0
Control		100	13.00	0.0	0.0
Pierced Control		200	35.00	0.0	0.0

*Significantly different from solvent $p \leq 0.05$

MANNITOL
AIR CELL 96 HOURS

DOSE mg/egg	DOSE mg/kg	Number of Eggs	Percent * Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	100	24.00*	0.0	0.0
5.00	100.00	100	16.00	0.0	0.0
0.75	15.00	100	13.00	0.0	0.0
0.375	7.50	100	12.00	0.0	0.0
0.1875	3.75	100	9.00	0.0	0.0
Water		100	11.00	0.0	0.0

*Significantly different from solvent $p \leq 0.05$

MANNITOL
YOLK 0 HOURS

DOSE mg/egg	DOSE mg/kg	Number of Eggs	Percent * Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	100	89.00 *	0.0	0.0
5.00	100.00	99	61.61	0.0	0.0
0.75	15.00	99	58.58	0.0	0.0
0.375	7.50	100	56.00	0.0	0.0
0.1875	3.75	100	45.00	0.0	0.0
Water		100	50.00	0.0	0.0

*Significantly different from solvent $p \leq 0.05$

MANNITOL
YOLK 96 HOURS

DOSE mg/egg	DOSE mg/kg	Number of Eggs	Percent * Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	100	66.00 *	1.0	1.0
5.00	100.00	100	64.60 *	0.0	0.0
0.75	15.00	100	52.00	0.0	0.0
0.375	7.50	100	44.00	0.0	0.0
0.1875	3.75	100	38.00	0.0	0.0
Water		100	38.38	0.0	0.0

* Significantly different from solvent $p \leq 0.05$